**Signosis**
Innovative Plate Assay Solutions

# Human Angiogenesis ELISA Plate Array

**Catalog Number EA-4012** **(For Research Use Only)**

## Introduction

Angiogenesis shifted from the avascular to vascular state is a key event for sustained tumor growth and cancer progression. Angiogenesis as a biological switch process is governed by numerous pro- and anti-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGFb), epidermal growth factor (EGF), and transforming growth factor-beta (TGFβ). The mechanism of action of each of these factors is different, as are their origin and the stimuli for their production. The angiogenic switch refers to the balance between pro- and anti- angiogenic factors. Therefore, profiling of these factors is critical to understanding angiogenesis. Signosis' Human Angiogenesis ELISA Strip Profiling Assay simultaneously profiles 16 human angiogenesis cytokines; TNFα, IGF-1, VEGF, IL-6, FGFb, TGFβ, EGF, Leptin, PDGF-BB, PlGF-1, β-NGF, SCF, MCP-1, MIP-1α, IL-2, and IL-4. The difference of these proteins between samples can be determined through data comparison.

## Principle of the assay

The 96-well clear plate is divided into 6 sections, and each section has 2 strips for one sample. In each section, 16 of specific cytokine capture antibodies are coated on 16 wells respectively, and one well without coating any antibody is used as a blank well. The sample, such as cell culture supernatants, cell lysates, tissue homogenates, serum, or plasma samples is incubated with cytokine ELISA plate, the captured cytokine proteins are subsequently detected with a cocktail of biotinylated detection antibodies. The test sample is allowed to react with pairs of two antibodies, resulting in the cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentrations of the cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

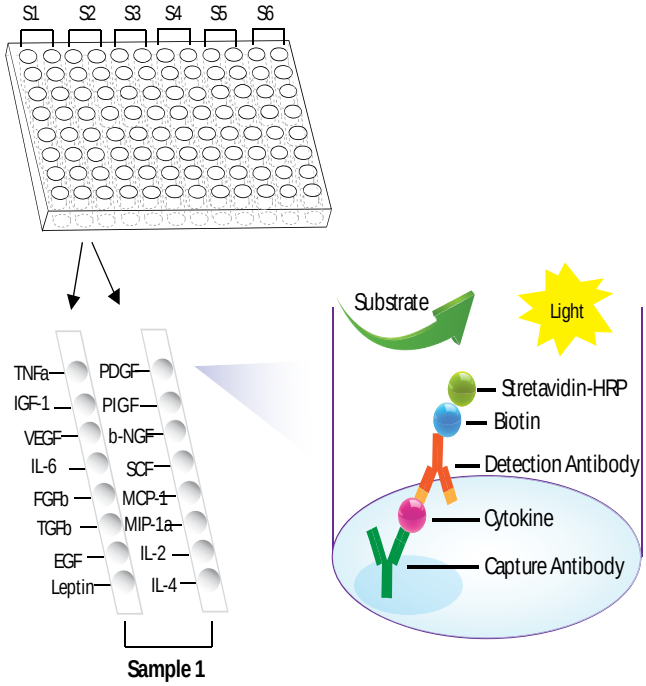


Diagram of Human Angiogenesis Plate Array

## Materials provided with the kit

| Component | Qty | Store at |
|---|---|---|
| **One clear plate coated with 16 different antibodies against human angiogenesis cytokines** | 1 | 4°C |
| **Biotin labeled antibody mixture against 16 different human angiogenesis cytokines** | 200 µL | -20°C |
| **Streptavidin-HRP conjugate** | 50 µL | 4°C |
| **1xDiluent buffer** | 40 mL | 4°C |
| **5X Assay wash buffer** | 40 mL | 4°C |
| **Substrate** | 10 mL | 4°C |
| **Stop solution** | 5 mL | 4°C |

## Material required but not provided

- Microplate reader
- Distilled H2O

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O.
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1x Diluent buffer.

## Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1x diluent buffer, for example, add 80 ul sample in 720 ul 1x diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

## Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare 1.7 ml sample and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
   ***Optional: If you want to have a blank reading, you can designate one well as a blank well by adding diluent buffer instead of your sample.***
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200 µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100 µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100 µl substrate to each well and incubate for 30-40 minutes at least.

   **Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes.**

9. Add 50 µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Human Angiogenesis ELISA Plate Array

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | TNFα | PDGFBB | TNFα | PDGFBB | TNFα | PDGFBB | TNFα | PDGFBB | TNFα | PDGFBB | TNFα | PDGFBB |
| B | IGF-1 | PlGF-1 | IGF-1 | PlGF-1 | IGF-1 | PlGF-1 | IGF-1 | PlGF-1 | IGF-1 | PlGF-1 | IGF-1 | PlGF-1 |
| C | VEGF | β-NGF | VEGF | β-NGF | VEGF | β-NGF | VEGF | β-NGF | VEGF | β-NGF | VEGF | β-NGF |
| D | IL-6 | SCF | IL-6 | SCF | IL-6 | SCF | IL-6 | SCF | IL-6 | SCF | IL-6 | SCF |
| E | FGFb | MCP-1 | FGFb | MCP-1 | FGFb | MCP-1 | FGFb | MCP-1 | FGFb | MCP-1 | FGFb | MCP-1 |
| F | TGFβ | MIP-1α | TGFβ | MIP-1α | TGFβ | MIP-1α | TGFβ | MIP-1α | TGFβ | MIP-1α | TGFβ | MIP-1α |
| G | EGF | IL-2 | EGF | IL-2 | EGF | IL-2 | EGF | IL-2 | EGF | IL-2 | EGF | IL-2 |
| H | Leptin | IL-4 | Leptin | IL-4 | Leptin | IL-4 | Leptin | IL-4 | Leptin | IL-4 | Leptin | IL-4 |